Chapter One

Die-back of cold-tolerant eucalypts associated with *Phytophthora* spp. in South Africa: A literature review

1 Introduction

The genus *Eucalyptus* (commonly known as 'eucalypts' or 'gum trees'), belongs to the large plant family *Myrtaceae* which consists of at least 700 species (Brooker 2000). The majority of the species are endemic to Australia with an exception of four species which are found in neighbouring Australasian islands (Ladiges *et al*. 2003). New species continue to be discovered and more are likely to be described in the future as robust molecular techniques become available to discriminate between similar sorts (Hill & Johnson 2000, Hill *et al.* 2001). Despite extensive review and taxonomic revision the taxonomy of *Eucalyptus* remains incomplete (Griffin *et al*. 1988), *Angophora* and *Corymbia* were included as subgenera (Brooker 2000). However, this was critised by Ladiges & Udovicic (2000). A view, consistent with both morphological (Hill & Johnson 1995) and molecular studies (Ladiges *et al*. 1995, Sale *et al*. 1993, Udovicic *et al*. 1995, Steane *et al.* 1999), suggests that in broad terms the eucalypts consist of three major lineages namely, the *Angophora* and *Corymbia* (bloodwoods, ghost gums) lineage and the *Eucalyptus sensu stricto* lineage. The latter comprises three major subgroups, namely, the *Symphyomyrtus*, *Eudesmia,* and *Monocalyptus* (Hill & Johnson 1995, Ladiges *et al*. 1995).

Eucalypts are versatile trees that grow in a variety of climatic conditions and in a diverse range of habitats (Turnbull 2000). They were initially introduced into the tropical and subtropical regions of Africa and South America for fuel wood, windbreaks, and land reclamation purposes since the 1900s (Poynton 1979, Evans 1982, Potts & Pederick 2000). Over the years, eucalypts have become a major source material for diverse commercial forest products such as pulpwood, fibreboard, sawn timber, poles, mine timber props, charcoal, honey and essential oils (Sedjo 1999, Turnbull 1999). A combination of biological factors such as wood properties, high productivity and versatile ecological adaptation have made eucalypts the most widely planted hardwood trees in many parts of the world (Turnbull 2000). As such, eucalypts are grown as a plantation species in several countries in Africa, Asia, South America and New Zealand, as well as throughout its extensive natural range in Australia (Eldridge *et al.* 1994). Eucalypt plantations are rapidly expanding and the total area under plantation is estimated to be at least 18 million hectares in 90 countries (Carle *et al*. 2002, FAO 2000).

High-yielding and genetically improved *E. camaldulensis*, *E. grandis, E. globules*, *E. urophylla*, and their inter-specific hybrids are leading sources of hardwood throughout the world (Campinhos 1999).

South Africa has a long history of planting eucalypts, since it does not have its own natural timber resources (Zwolinski & Bayley 2001). It is amongst the leading *Eucalyptus* growing countries in the world (Schönau *et al*. 1994, Owen & van der Zel 2000). These plantations are distributed along the eastern coastline of South Africa, and comprise an assortment of species and hybrids that are planted in various habitats with diverse climatic conditions (Forestry South Africa 2008). Eucalypts in South African plantations are harvested at short rotation periods of 7-8 years for production of pulpwood and other timber products (Poynton 1981). Because of its suitability for such purposes, improved *E. grandis* and its various clones, crosses and hybrids are the most commonly planted genotypes (Wingfield *et al*, 2002). These constitute more than 75% of commercial eucalypt plantings in South Africa, and the total area planted to *E. grandis* is at least 290, 155 ha (Forestry South Africa 2008). *E. grandis* is a preferred forest tree species for commercial forestry in subtropical areas because it grows fast, has good form and versatile wood and pulp properties (Turnbull & Pryor 1978). It is amongst the best *Eucalyptus* spp. for short rotation forestry and is also planted extensively in South America (Eldridge *et al.* 1994). *E. grandis* performs very well and produces good yield per hectare when planted at correct sites (Eldridge *et al.* 1994). However, it grows slowly or even fails to establish if planted at cold or frost prone sites (Swain & Gardner 2003). Consequently, for plantations in such areas foresters are forced to make use of species or hybrids with great tolerance of low temperatures (Swain & Gardner 2003).

Cold-tolerant eucalypts were first introduced into South Africa at the turn of the 20th century for the production of mine roof support columns (Poynton 1979). Afforestation using cold-tolerant gums has increased steadily over the past decade due to increased demand for pulp and paper, promoting the rapid expansion of forestry into marginal or high altitude areas that are unsuitable for *E. grandis* (Edwards, 2000, Swain *et al*. 2000, Swain & Gardner 2003). Today cold-tolerant eucalypts cover a total area of more than 201,779 hectares in South Africa (Forestry South Africa 2008). Cold-tolerant *Eucalyptus* spp. commonly planted include *E. dunnii*, *E. nitens* and *E. macarthurii* (Darrow 1984). Less commonly planted eucalypts include *E. cloeziana, E. elata*, *E. fastigata, E. saligna* and *E. smithii.* While these species enjoy an advantage over *E. grandis* in terms of cold tolerance, their commercial value is limited by inferior pulp and fibre quality (Clarke *et al*. 1999). Hence, there is a need to find alternative cold-tolerant eucalypts suitable for high altitude areas in South Africa (Little & Gardner, 2003). *E. fraxinoides*, *E. smithii* and *E. oreades* are cold-tolerant with a potential for commercial propagation in such areas (Clarke *et al*. 1999). They grow vigorously and produce high quality pulp and paper compared to other *Eucalyptus* spp., including *E. grandis* (Clarke 1995, Clarke *et al*. 1999). However, they have an important shortcoming. This is that they are all susceptible to root and collar rot (Linde *et al*. 1994a, Wingfield & Roux 2000). A number of soilborne pathogens

belonging to the genus *Pythium* and *Phytophthora* have been reported to cause this disease. The following section presents an overview of the genus *Phytophthora* and its taxonomic history. This is followed by a description of *Phytophthora* die-back among eucalypts in plantations and strategies employed to manage this disease. The development of effective disease management strategies depends on a comprehensive understanding of the biology and epidemiology of the pathogen, which in turn requires insight into the genetic variation between and within *Phytophthora* spp. This review concludes, therefore, with a discussion of morphological and non-morphological methods used to distinguish between species, sub-species, and varieties within the genus.

2 Overview of the genus *Phytophthora*

The genus *Phytophthora* belongs to a diverse and primitive group of 'fungus-like' mycelial organisms, commonly referred to as "water moulds" (Agrios 1997). In 1996 there were approximately 54 described species (Erwin & Riberio 1996). The number of described species and designated taxon has increased rapidly in the past 10 years due to molecular identification of cryptic species and large scale environmental surveys in natural ecosystems and there are now over 105 recognised species and taxa (Brasier, 2008). Its name (Greek: *Phyton*, plant + *phtheiro*, destroyer) derives from the fact that it includes some of the most destructive plant pathogens (Judelson & Blanco 2005). The type species, *P. infestans*, destroyed Ireland's potato crop leading to a famine during the 19th century (Gregory 1983, Bourke 1991). Even today late blight is active and widespread and is responsible for high losses in potato production in many parts of the world (Duncan 1999).

Phytophthora spp. have long been classified as prokaryotic fungi under the Order Pythiales and Phylum Oomycota (Waterhouse 1973) because they are physiologically and morphologically similar to true fungi (Erwin & Riberio 1996). However, they represent a distant evolutionally line (Dick 1990a, 1997, Barr 1992, Cavalier-Smith 1998) and unique biological features distinct from true fungi (Erwin *et al.* 1983). For example, the genus *Phytophthora* differs from true fungi by having a diploid life cycle, non-septate mycelia, different cell wall and membrane structure, a requirement for external source of sterols for sporulation and thiamine for growth and different pathways for synthesizing lysine (Hendrix 1970, Bartnicki-Garcia & Wang 1983, Elliott 1983). Oomycetes including members of the genus *Phytophthora* have been reclassified under a recently described kingdom Stramenopila (or Chromista) (Cavalier-Smith 1998, Yoon 2002) based on their close evolutionary relationship with biflagellate, heterokont brown algae (Chromophyte algae), which possess tinsel-like flagellae (Gunderson *et al.* 1987, Förster *et al.* 1990a, Brasier & Hansen 1992, Paquin *et al.* 1995). However, according to Barr (1992) the similarity between oomycetes and algae was first postulated in 1858 by Pringsheim and this hypothesis is supported by sequence data of the small subunit of the RNA (Ariztia *et al*. 1991, Wainright *et al.* 1993). Recent analysis of the nuclear large subunit ribosomal DNA sequence data indicate that the genus *Phytophthora* is more closely related to the genera

Peronophythora, *Bremia* and *Plasmopara* and not *Pythium* as previously thought (Riethmüller *et al*. 2002). Although oomycetes, including *Pythium* and *Phytophthora,* are unrelated to fungi, Dick (1997) and Money (1998) recommends that the Oomycetes should be, for practical purposes, still be treated as fungi. Therefore, for the purpose of this review, the convention term(s) "fungi or fungal-like" are used to refer to *Phytophthora*.

2.1 Disease cycle of *Phytophthora*

Phytophthora spp. produce motile biflagellate zoospores that differentiate within the sporangium. When abundant water is available, zoospore move upward (negative geotaxis) towards the surface of the soils and are chemically attracted to roots of the host plant and readily initiate infection (Zentmyer 1961, Hinch & Weste 1979, Carlile 1983). The zoospores encyst and germinate to produce germ tubes, which infect and penetrate fine roots and progress to larger roots (Shearer *et al*. 1981, Zentmyer 1980).

Phytophthora spp. can either be homothallic or heterothallic. The heterothallic species produce thick walled oospores (sexual spores) when fertilisation of two opposite compatible mating types (A1 and A2) occurs. The homothallic species have the ability to form oospores independently without crossing. Oospores form one or more germ tubes, which initiate mycelial growth, or sporangia that produce either zoopores or chlamydospores. Oospores and chlamydospores can survive in the soil for many years (Duncan 1980, Duncan & Cowan 1980). Limited information is available about the dormancy of oospores and chlamydospores and the factors that activate their germination.

2.2 Taxonomic history of the genus *Phytophthora*

The history of the taxonomy of the genus *Phytophthora* began with the description of *P. infestans* by Anton de Bary in 1876 (Erwin & Riberio 1996). According to historical accounts by Erwin & Riberio (1996), in 1931 Tucker produced the first monograph, based on 21 species, and proposed a classification system based on morphology, physiology and pathology. In 1963, Waterhouse produced a taxonomic key in which 41 species were divided into six groups based on the shape and size of sporangia and attachment of antheridia to the oogonium. However, the taxonomic relevance of some morphological characteristics such as the position of antheridia has been challenged recently by Hüberli *et al*. (1997) and Gao *et al*. (1998). The revised tubular keys of Newhook *et al*. (1976), Stamps *et al*. 1990 and Erwin & Riberio (1996) were used for many years as traditional references for the

taxonomy and systematics of more than 50 *Phytophthora* species. Other taxonomic keys specific to countries such Taiwan and South Africa were published (Wager 1931, 1941, Ho *et al*. 1995).

Presently, the number of described *Phytophthora* spp. has increased rapidly and recently the taxonomy of the genus *Phytophthora* underwent major revision (Cooke *et al.* 2000, Kroon *et al*. 2004). This is mainly due to improved molecular techniques (Lévesque *et al*. 1998, Drenth *et al*. 2006, Bailey *et al.* 2002). However, other factors such as nursery trade of plant material and their associated pathogens across countries have been reported to play a role (Wingfield *et al*. 2001, Brasier *et al*. 2004, Brasier 2008). In 1996 less than 20% of *Phytophthora* species were known from forests and natural ecosystems (Brasier 2008, Erwin & Riberio 1996). Since 2000, over 50 new species have been described or are under description. The majority of these new species are from forest ecosystems (Brasier 2008).

3 Impact of *Phytophthora* diseases

The range of plants attacked by members of the genus *Phytophthora* spp. is enormous. Species such as *P. cinnamomi*, *P. nicotianae* and *P. cactorum* have a very broad host range, although others such as *P. infestans* and *P. sojae* are restricted to few host plants (Zentmyer 1983). Much damage is caused by these pathogens to native host species in natural environments for example in Western Australia, large areas of the native jarrah (*Eucalyptus marginata*) forest have been destroyed by *P. cinnamomi* (Podger *et al*.1965, Newhook & Podger 1972, Podger 1972, Weste & Marks 1987, Shearer & Tippett 1989, Shearer *et al.* 2004) as well as more recent outbreaks in other parts of the world (Tainter *et al.* 2000, Gallego *et al.* 1999). In addition, this pathogen also attacks a number of rare, susceptible Australian endemic species such as *Banksia* spp. (Shearer & Dillon 1995, 1996, Peters & Weste, 1997, Scott *et al*. 2009, Burgess *et al*. 2009). According to published reports, more than 2000 plant species are susceptible to *P. cinnamomi* in South-Western Australia alone (Wills 1993, McDougall *et al*. 2001, 2005).

There is an increasing concern regarding the impact of *Phytophthora* on food and wood fibre production worldwide. The recently described *P. ramorum* previously known only from two countries in Europe on *Rhododendron* and *Viburnum* killed large numbers of native *Quercus* spp. in California and Oregon and has jumped to other hosts such as redwoods in the United States (Rizzo *et al.* 2002, Tooley & Kyde 2007, Grünwald *et al*. 2008). It is evident from the above examples that plant disease epidemics caused by invasive pathogens such as *P. cinnamomi* can cause widespread death and decline of several susceptible native plant and tree hosts.

Hybridization between different *Phytophthora* spp. is possibly resulting in new host specific species (Brasier *et al*. 1990, 1995, Ioos 2006). Several new and often aggressive *Phytophthora* spp. continue to be discovered in Europe (Jung *et al.* 1999, 2002, 2003, Brasier *et al.* 2004, 2005) and other parts of the world (Greslebin *et al*. 2007, Maseko *et al* 2007, Durán *et al.* 2008, Scott *et al*. 2009), resulting in expanded lists of susceptible hosts (Mirabolfathy *et al.* 2001, Fier *et al.* 2002, Polashock *et. al*. 2005). These recent discoveries reflect an increasing threat posed by these pathogens in native and plantation forests. As knowledge is increased and molecular diagnostic tools are refined, new species will be discovered.

In South Africa, *Phytophthora* related diseases are widespread in several agricultural crops, ornamentals and horticulture, forest tree species. More than 15 *Phytophthora* spp. have been reported from 74 different hosts in Southern Africa (Crous *et al*. 2000, 2006). Susceptible agricultural and ornamental crops include *Persea americana* (Darvas *et al*. 1987), *Musa acuminata* (Thompson 1981), *Gypsophila paniculata* (Thompson & Naudé 1992), *Brassica oleracea* (Thompson & Phillips 1988), citrus (Thompson *et al*. 1995), *Vitis vinifer*a (Marais 1979, 1980 Halleen *et al*. 2003), *Medicago falcata* (Botha 1993), *Nicotiana tabacum* (van Jaarsveld 2001), *Lycopersicom esculantum* (Ferreira *et al*. 1991) and *Allium cepa* (von Maltitz & von Broembsen 1984). Susceptible exotic forest tree species include black wattle (*Acacia mearnsii*) (Zeijlemaker 1971), *Pinus* and *Eucalyptus* spp. (Donald & von Broembsen 1977, Darvas *et al*. 1978, Wingfield & Knox-Davies 1980). Indigenous tree hosts susceptible to *Phytophthora* root rot include *Leucadendron argenteum* (van Wyk 1973), *Ocotea bullata* (von Broembsen *et al*. 1986, Lubbe & Geldenhuys 1990, Lubbe & Mostert 1991) and the Clanwilliam cedar (*Widdringtonia cedarbergensis)* (Wingfield *et al.* 1988). *Phytophthora cinnamomi*, in particular is a serious treat to the several exotic hosts and indigenous flora (fynbos) endemic to the South Western Cape (von Broembsen 1984). However, very little is known about the interaction between *Phytophthora* spp., and native forest species in South Africa. Whereas, the interaction of *P. cinnamoni* and native forests has been extensively studied in Australia (Weste & Marks 1987, Shearer *et al.* 2000, Shearer & Tippett 1989). The Western Cape Province of South Africa and Western Australia share the mediterranean climate and closely related vegetation type including, *Proteaceae* family (*Protea* and *Banksia*), which are reported to be highly susceptible to *Phytophthora* root rot (Burrows 1985, von Broembsen & Brits 1985). Thus, the potential threat of *P. cinnamoni* to the indigenous fynbos vegetation of the south Western Cape still requires extensive research.

Diseases of exotic forest species associated with *Phytophthora* in South Africa have been the subject of several recent reviews (Linde *et al.* 1994a, Roux *et al*. 1995). *P.nicotianae* is mainly associated with black butt of black wattle (Roux *et al*. 1995). Other known pathogens associated with this disease include include *P. boehmeriae* and *P. meadii* (Roux *et al*. 1997). *P. cinnamoni* is the main causal agent of root and collar rot of cold-tolerant eucalypts in South Africa (Linde *et al*. 1994b). However, *P. boehmeriae* and *P. nicotianae* have also been recorded as causal agents of eucalypt collar and root rot

include (Linde *et al*. 1994b, Maseko *et al.* 2001). In the past, *Phytophthora* spp. were mostly identified by examining morphological features but more recently molecular techniques have been employed.

3.1 *Phytophthora* die-back of eucalypts

A number of *Phytophthora* spp., have been reported as serious soil-borne pathogens associated with native eucalypts (Newhook & Podger 1972, Pratt & Heather 1973, Fagg 1987, Shearer *et al.* 1987a) and in eucalypt plantations in many countries (Shearer & Smith 2000). Some infect young eucalypt seedlings in nurseries leading to pre-emergence damping-off and post-emergence premature death of seedlings leading to serious losses of planting stock (Marks & Kassaby 1976, von Broembsen 1984, Bayley & Snell 1997, Brown & Ferreira 2000). In addition, *Phytophthora* spp. also cause root and collar rot disease of older seedlings in plantations leading to poor growth and in some cases early death (Shearer & Smith 2000). In Australia and South Africa, collar rot of eucalypts associated with *Phytophthora* spp. in mining rehabilitation and plantation sites is confined to areas, which are often subject to occasional waterlogging (Jarvel 1998, Colquhoun & Elliot 2000, Colquhoun & Hardy 2000).

Although, a number of *Phytophthora* spp. have been isolated from eucalypt forests, *P. cinnamoni* is considered the single most destructive pathogen associated with eucalypts and their associated understorey species in Western Australia (Shearer & Smith 2000). In contrast, *P. cinnamoni* seldom causes diseases of eucalypt in nurseries and plantations in Australia and in several eucalypt growing countries because resistant eucalypts are usually planted (Brown & Ferreira 2000). In South Africa, for example, the commonly grown *E. grandis* is less prone to *Phytophthora* root rot in comparison to the susceptible cold-tolerant eucalypts (Wingfield & Knox-Davies 1980). The role of other *Phytophthora* spp., in native and plantation forests in less known, however, their potential role as serious pathogens is becoming more significant as they are associated with new disease outbreaks in *Eucalyptus* nurseries (Belisario 1993) and in plantations (Maseko *et al.* 2001).

3.1.1 *Phytophthora* related diseases in *Eucalyptus* nurseries

Nursery propagation of eucalypt seedlings is the key phase of any eucalypt-planting programme (Eldridge *et al*. 1994). Losses of plating stock due to *Phytophthora* related diseases can thus severely affect a planting programme (Brown 2000). The nursery conditions in general are conducive to the development of disease epidemics caused by *Phytophthora* spp. Over watering, poor drainage and high seedling density are some of the main factors contributing to the rapid spread of waterborne pathogens in nurseries (Old *et al*. 2003). Eucalypt clones grown in hydroponic nurseries in particular,

are even more vulnerable to *Phytophthora* related disease outbreaks (Lombard 2004), especially if untreated water is used.

Damping-off is one of the most important nursery diseases caused by a wide range of soil borne pathogens including *Phytophthora* spp. (Brown & Ferreira 2000). They are involved in pre-emergence and post-emergence damping-off disease affecting eucalypts and also cause root and collar rot diseases of older seedlings (Gibson 1975, Brown & Ferreira 2000). *P. cinnamomi* and *P. nicotianae* are common nursery pathogens found in forest nurseries in South Africa (Lombard 2004). There are, however, few reports of nursery diseases attributed to *Phytophthora* spp. in South Africa. Damping-off was once considered to be the most important disease of exotic forest trees including *Eucalyptus* seedlings in nurseries (Lückhoff 1964, Heather *et al*. 1977, Darvas *et al.* 1978, von Broembsen 1984). However, most outbreaks were consistently traced to poor nursery practices (Brown 2000). Damping-off has also been a problem in the propagation of eucalypt seedings in nurseries in Australia in the past and *Phytophthora* and *Pythium* spp. were implicated in plant death (Brown & Wylie 1991).

3.1.2 *Phytophthora* related disease symptoms in *Eucalyptus* plantations

Eucalypt plantations differ from natural forests in a number of respects. The latter consist of a variety of species and thus are more complex than man-made forests (Gadgil *et al.* 2000). *Eucalyptus* plantations usually consist of a single species planted on an industrial scale. *Eucalyptus* plantations represent monocultures, especially where the trees are clonally propagated and they are consequently more vulnerable than native forests to invasion by pests and pathogens (Wingfield *et al*. 1991, Gadgil & Bain 1999). The rapid turnover cycle also allows a build up of pathogens because the soil ecology is in a constant state of disturbance.

In South Africa, *P. cinnamomi*, a pathogen believed to be introduced (Linde *et al.* 1997) into South Africa, is the most destructive and aggressive pathogen associated with die-back of exotic eucalypts (Wingfield & Roux 2000). However, recently *P. nicotianae* rather than *P. cinnamomi* was consistently isolated from dying cold-tolerant eucalypts (Maseko *et al.* 2001). *P. nicotianae* also causes the disease known as black butt on the non-native *Acacia mearnsii* de Wild in South Africa (Zeijlemaker 1971, Roux & Wingfield 1997).

The initial symptoms of *Phytophthora* die-back occur below ground and thus often go unnoticed because *Phytophthora* spp. do not form visible fruiting structures in infected tree host. The expression of advanced disease symptoms such as wilting and die-back in eucalypts are observed long after the initial infection has taken place (Shearer & Tippett 1989). Infected root collars and roots usually have

water-soaked discoloured lesions, although asymptomatic infection of *P. cinnamoni* in *E. marginata* has been demonstrated (O'Gara *et al.* 1996, 1997, Hüberli *et al*. 2000). The most obvious above ground disease symptoms associated with eucalypts as the result of *Phytophthora* infection is a gradual wilting of the leaves resulting from girdling of the root collar leading to canopy thinning, die-back and ultimately death of the tree. If the bark is removed, brown lesions, extending from the roots are often observed. Other disease symptoms include root rot, gum exudation from diseased tissue and the formation of epicormic shoots. Death and poor growth of eucalypts in plantations is localised and is characterized by the presence of patches of dead trees. Reports suggest that eucalypt die-back is complex in nature and is possibly caused by a number of pathogens including *Phytophthora* and *Pythium* spp. (Linde *et al.* 1994b, Wingfield *et al.* 2001).

3.1.2 Physiological and anatomical response to bark injury

Infection of eucalypts by *P. cinnamomi* induces a series of physiological responses in the host. The initial physiological responses are stimulation of defence mechanisms aimed at preventing the spread of the pathogen (Dixon *et al*. 1994). Tolerance to *Phytophthora* spp. in eucalypts is associated with the ability of the host to limit colonization and necrosis of the infected tissue (Byrt & Holland 1978, Cahill *et al.* 1989). Resistance of eucalypts is often expressed by the inhibition of *Phytophthora* spp. in secondary root tissue (Tippett *et al*. 1985). Accumulation of secondary plant metabolites such as lignin and phenolic compounds are usually associated with this type of reaction in eucalypts (Tippett & Malajczuk 1979, Cahill *et al*. 1993). The formation of the wound induced periderm tissue in the secondary tissue of eucalypts also plays an important role in resistance (Tippett *et al.* 1983, Tippett & Hill 1984).

A second suite of physiological responses by the host occurs when *Phytophthora* spp. invade roots and prevent the uptake and absorption of water and minerals (Cahill *et al*. 1986, Tippett *et al*. 1987, Weste & Marks 1987). Responses include production of tyloses (Dawson & Weste 1984), increased respiration and cell wall permeability, and leakage of electrolytes (Cahill & Weste 1983, Cahill *et al*. 1985b). Increased production of tyloses has also been associated with waterlogging in *E. marginata* seedlings (Davison & Tay 1987). The increase in nutrient exudates and production of tyloses following infection by *Phytophthora* spp. is greater on susceptible than on tolerant eucalypts (Weste & Marks 1987).

Secondary metabolites such as glucans are secreted by some *Phytophthora* spp. and have been reported to induce wilting in eucalypts (Halsall 1978). However, glucan is not associated with the pathogenicity of *Phytophthora* spp. (Cahill *et al*. 1985a). *Phytophthora*-mediated hormonal imbalance

is also suspected to play a major role in the development of symptoms in susceptible eucalypts (Cahill *et al*. 1985a).

3.1.3 Variation of disease susceptibility amongst *Eucalyptus* spp.

Several studies have shown that eucalypts differ significantly in their response to infection by *Phytophthora* spp., especially *P. cinnamomi* (Weste & Taylor 1971, Marks *et al.* 1972, Marks *et al*. 1973, Pratt *et al.* 1973, Tippett *et al.* 1985). *Eucalyptus* spp. belonging to the subgenus *Monocalyptus* are more susceptible to *P. cinnamomi* than species which belong to the subgenus *Symphyomyrtus* or the genus *Corymbia* (Weste & Marks 1974, Noble 1989). In South Africa, *E. fastigata (Monocalyptus)* has been reported to be susceptible to *P. cinnamomi* while *E. grandis (Symphyomyrtus)* is tolerant (Wingfield & Knox-Davies 1980). During the 1980s, *Phytophthora* disease outbreaks led to the termination of the commercial propagation and planting of *E. fastigata* in South Africa (Eldridge *et al.* 1994). *E. fastigata* has since been gradually replaced by species more tolerant to infection by *Phytophthora* spp. (Linde *et al.* 1994a).

Significant variation in tolerance to *P. cinnamomi* appears to exist amongst susceptible *Monocalyptus* subgenera. Evidence from greenhouse inoculation studies have shown that certain individual trees within the susceptible *E. marginata* half-sib families exhibit some degree of tolerance to *P. cinnamomi* (Stukely & Crane 1994). Variation in tolerance to *P. cinnamomi* has also been demonstrated on *E. regnans* F. Muell (Marks *et al.* 1981, Harris *et al*. 1985) and on *E. marginata* clones (Cahill *et al*. 1992, Bennett *et al*. 1993, Hüberli *et al*. 2002a, 2002b).

3.1.5 Variation in pathogenicity among *Phytophthora* isolates

Pathogenicity refers to the ability of a pathogen to cause a disease in a specific host (Bos & Parlevliet 1995), while virulence refers to the degree of pathogenicity (Agrios 1997). Shaner *et al.* (1992) employs a more precise definition of virulence, using the term to denote the genetic ability of a pathogen or race to overcome genetically determined host resistance. In this review, however, the term "virulence" will not be used in this sense because virulence genes have not been clearly defined for *Eucalyptus.*

Selection and breeding for tolerance to *Phytophthora* spp. in susceptible *Eucalyptus* spp. must take into account the pathogenicity of the *Phytophthora* spp. involved (Shearer *et al.* 2000). In view of this fact, comprehensive studies on the variation of *P. cinnamomi* isolates on eucalypts have been conducted in Australia (Dudzinski *et al.* 1993, Hüberli 1995, Hüberli *et al.* 2001b) and in South Africa

(Linde *et al.* 1999b, Linde *et al.* 2001, Robin *et al*. 1998). There are several reports on the pathogenicity of *P. cinnamomi* and other *Phytophthora* spp. on eucalypts (Marks & Kassaby 1976, Shearer *et al*. 1988) and other forest tree hosts (Weste 1975, Hamm & Hansen 1982, Hansen *et al*. 1999, Jung *et al.* 1999). However, very little is known about the interaction of the combined effect between *P. cinnamomi* and other *Phytophthora* spp. in forest ecosystems where they coexist.

3.1.6 Assessment host tolerance and pathogenicity of *Phytophthora* spp.

Pathogenicity tests have been conducted on young eucalypts in tissue culture (Cahill *et al.* 1992), in pot trials under greenhouse conditions (Butcher *et al*. 1984, Marks *et al.* 1972, Maseko 1999) and in the field on young trees (Marks *et al.* 1981, Wolfaardt *et al.* 1997, Linde *et al.* 1999b, Linde *et al.* 2001). These tests are used to distinguish between eucalypts susceptible or tolerant to *Phytophthora* spp. Excised stems from eucalypts and other susceptible hosts have also been used in determining the pathogenicity of *P. cinnamomi* isolates (Dixon *et al*. 1984, Lundquist & Baxter 1985, Shearer *et al.* 1987a, Gabor & Coffey 1991, Tynan *et al*. 1998, Hüberli *at al*. 2002b). From these studies it is evident that there is great variation in pathogenicity among *P. cinnamomi* isolates.

In wound inoculation studies, variation in resistance to *Phytophthora* is assessed by the degree of lesion extension (Marks *et al.* 1981). Although this method has been widely used in the past, it has shortcomings as it bypasses the bark that is a natural barrier to pathogen invasion. Studies by O'Gara *et al*. (1996, 1997) and Lucas *et al.* (2002) have shown that *P. cinnamomi* does not require wounds to infect *E. marginata.* The extent to which the bark of trees is able to withstand infection, therefore, presents a source of variation in their resistance to *Phytophthora* that is not taken into account by wound inoculation studies.

4 Role of environmental factors in the development of *Phytophthora* die-back on eucalypts

Several stress-inducing factors influence the growth and development of eucalypts in indigenous forest and plantations. These factors include biotic and abiotic agents, and can predispose trees to infection by *Phytophthora* spp. Biotic factors include other pathogens and pests. Abiotic factors, on the other hand, include the health and nutritional status of eucalypts as well as extremes in temperature and soil moisture, which are discussed in detail below.

4.1 Soil Moisture

The life-cycle of soil borne *Phytophthora* spp. is largely dependent on moisture, which influences development in soil as well as in host tissue (Smith & Marks 1982, Tippett *et al.* 1987, Bunny *et al*. 1995). Different moisture conditions affect the pathogen and host in different ways, and their impact

on the likelihood of *Phytophthora* infection is sometimes complex. It is useful to emphasize two moisture conditions: surface ponding and waterlogging. In the former, the oxygen content of water is typically high. In the latter, soil is saturated with water creating hypoxic and potentially anoxic conditions, especially in poorly drained soils.

Surface ponding provides an ideal opportunity for *Phytophthora* collar infection because it wets the bark of the tree collars while high oxygen concentrations remain. Waterlogging, by contrast, has been reported to inhibit the activity of *P. cinnamomi* as this species cannot sporulate under low oxygen conditions (Burgess *et al.* 1998). However, waterlogging also induces stressful conditions that harm eucalypts and predispose them to invasion by *Phytophthora* spp. (Newhook & Podger 1972, Duniway 1979). Waterlogging has an inhibitory effect on the growth and vigour of eucalypts, especially on poorly drained sites (Burgess *et al.* 1999), and the effects of waterlogging alone can be sufficient to cause die-back of *E. marginata* (Davison & Tay 1987).

The harmful effects of waterlogging on eucalypts in indigenous and plantations forests are well documented (Newhook & Podger 1972, Duniway 1983, Bell 1999, Niknam & McComb 2000). Eucalypts have been found to differ in their ability to tolerate waterlogged conditions. Species residing in the *Monocalyptus* for example, are more sensitive to waterlogging than species of *Symphyomyrtus* (Niknam & McComb 2000). The physiological and genetic mechanisms involved in waterlogging tolerance in eucalypts are not clearly understood.

4.2 Temperature

Temperature is one of the most important abiotic factors infuencing the development of *Phytophthora*-related diseases in plants. Seasonal variation in particular, determines the time of *Phytophthora* disease outbreaks, which are usually associated with wet soil conditions and moderate temperatures. For example, in Western Australia *Phytophthora* die-back in the jarrah forests is severe during winter and spring when both the temperature and soil moisture are highly favourable to *P. cinamomi* infection (Weste & Marks 1987).

All major components of the *Phytophthora* life cycle are influenced by seasonal variables such as temperature and moisture (Duniway 1983). Temperature directly influences the sporangia formation and germination, inoculum production and survival in the soil (Shearer & Tippett 1989). Thus temperature plays an important role in the epidemiology of the *Phytophthora*-related diseases since it influences the pathogen directly in the soil and within the host tissue (Shearer & Tippett 1989). The temperature-growth relationships of *P. cinnamomi* in culture and in secondary phloem tissue of *E. marginata* have also been studied in detail (Shearer *et al*. 1987b). In a study conducted by Grant and Byrt (1984), the extent to which host tissue is colonized was reported to be dependent on temperature

in both tolerant and susceptible eucalypts. Hüberli *et al* (2002a) shows how temperature affects the growth of *P. cinnamomi* within one host (cloned jarrah); as temperature got into the optimal levels for the pathogen, resistant clones were as susceptible as susceptible clones of jarrah.

4.3 Soil nutrition

Nutrition plays an important role in the growth and survival of eucalypts. The nutrition of eucalypts in their native environment and in plantations has been subjected to comprehensive reviews (Herbert 1992, Attiwill & Adams 1996). Nutrient deficiencies of eucalypts lead to reduced growth and increased susceptibility to diseases (Showdon 2000). Eucalypts growing on sites with poor nutritional status have been found to be more susceptible to *P. cinnamomi* (Tippett *et al*. 1989). Calcium, however, has been reported to enhance resistance of eucalypts towards *P. cinnamomi* in the field and in glasshouse conditions (Boughton *et al* 1978, Halsall 1980, Cahill *et al.* 1986).

5 Management of *Phytophthora* die-back in eucalypt plantations

Effective management of *Phytophthora* die-back is essential, as it is one of the destructive diseases in eucalypt-growing countries (Wingfield 1990, Gadgil *et al.* 2000). Prevention is essentially the basis of all management strategies used in *Eucalyptus* plantations because chemical control is not financially viable. Integrated disease management strategies involving quarantine, silvicultural practices and planting of disease resistant trees are currently used in eucalypt plantations.

5.1 Quarantine and sanitation

Quarantine and sanitation measures are effective disease control strategies used in many eucalypt nurseries and forest operations. They are aimed at preventing accidental introduction of a pathogens and pests into disease-free areas. A combination of three quarantine and sanitation methods is used in forest nurseries. These are pathogen eradication, reduction of pathogen propagules, and prevention of pathogen spread (Old *et al*. 2003).

Pathogen eradication measures include all practices aimed at eliminating pathogens from all nursery operations. Examples of such practices include decontamination of nursery equipment, pre-treatment of seedlings and seeds (Donald & Lundquist 1988) and the use of steam-sterilized trays (Donald *et al.* 1994, Myburgh 2000). Pathogen reduction methods are aimed at reducing the inoculum levels of the *Phytophthora* spp. in forest nurseries, and include the use of composted bark media (Hoitink *et al*. 1976, 1977, Hoitink 1980, Hoitink & Fahy 1986, Hardy & Sivasithamparam 1991). Measures aimed at preventing the spread of pathogens in forest nurseries include chemical and biological control.

Systemic fungicides such as metalaxyl and fosetly-Al are used to control *Phytophthora* root and collar rot in nurseries (Ali Smith & Guest 1999, Hardy *et al*. 2001, Wilkinson *et al.* 2001).

5.2 Silvicultural practices

Intensive management of eucalypts plantations through silvicultural strategies enhances tree growth and vigour and thus reduces the risk of diseases (Denison & Kietzka 1993). Silvicultural practices such as appropriate site preparation, timely fertilization, and weed control enhance seedling survival and vigour during establishment (Donald 1987, Gadgil *et al.* 2000). Eradication of pathogens through careful use of controlled burning; hygienic thinning and pruning are effective disease control strategies.

Correctly matching species to sites is a commonly used disease avoidance strategy. As was mentioned earlier, different eucalypts have different site requirements (Herbert 2000). When a *Eucalyptus* sp. is planted on unsuitable sites, it is prone to stress and damage by pathogens. Hence, the use of appropriate site and species selection can prevent or limit disease losses (Herbert 1994, Louw 1999).

5.3 Breeding and selection for disease resistance

Breeding for disease resistance is one of the most important aspects of any *Eucalyptus* improvement programme worldwide. Many such breeding programmes were started several decades ago with initial emphasis on improving phenotypic traits such as growth rate, stem form and wood density. *E. grandis* breeding programmes in Brazil and in South Africa serve as classic examples (van Wyk 1973, Eldridge *et al.* 1994). Originally, eucalypt breeding programmes in South Africa did not pay much attention to disease resistance. Due to the increased impact of fungal pathogens, however, this strategy is now an integral part of all eucalypt breeding programmes (Gadgil & Bain 1999, Gadgil *et al.* 2000). A measure of the success of such programmes is the fact that many eucalypt plantations remain disease free for several years until new pathogens are accidentally introduced from other countries or jump from native hosts (Gadgil *et al.* 2000).

Disease resistance in forest trees has been subjected to many reviews (Bingham *et al*. 1971, Carson & Carson 1989, Namkoong 1991). Significant advances have been made in controlling fungal disease through selection and breeding programmes of forest trees (Bazzigher 1981, Nomkoong *et al*. 1988, Smalley & Guries 1993). Successful screening techniques for tolerance to *P. cinnamomi* have been reported on pine (Butcher *et al.* 1984) and on eucalypts (Stukely & Crane 1994) and a good correlation between greenhouse and field trials have been achieved. Although complete resistance towards *P. cinnamomi* on susceptible eucalypts has not been achieved, planting of tolerant trees has proven successful enough to justify intensive selection efforts (Stukely *et al*. 2007).

5.4 Importance of maintaining genetic diversity

The importance of genetic diversity in forest tree species has been reviewed in the past (Namkoong *et al* 1998, Namkoong 1991). As was pointed out earlier, plantations are usually made up of a single species and are thus less diverse than mixed indigenous forests. This makes them more vulnerable to diseases (Chou 1981, Wingfield *et al.* 1991, Simpson Simpson & Podger 2000). Maintaining genetic diversity in planting stock minimizes the risk of serious losses due to diseases (Namkoong 1991, Wingfield *et al.* 2001).

5.5 Knowledge of the disease epidemiology

Sound knowledge of the biology of the pathogen is a prerequisite to any disease control strategy (Wingfield *et al.* 2001). The biology of *P. cinnamomi* in native Australian forests has been extensively studied (Shearer & Tippett 1989, Weste & Marks 1987), although little is known about the biology of other *Phytophthora* spp. in native forests. Population diversity studies are also helpful in determining the origins and reproduction strategies of pathogens (Linde *et al.* 1997, Dobrowolski *et al.* 2002, 2003). The epidemiology of pathogens plays a particularly important role in disease management because it has a direct impact on disease control strategies (Ristaino & Gumpertz 2000). Modern molecular techniques offer a powerful array of tools for studying these aspects of *Phytophthora* spp., and are discussed in the following section.

6 Identification of *Phytophthora* spp.

As has been mentioned earlier, taxonomic keys currently used for the identification of *Phytophthora* spp. are based on morphological groups, where each group encompasses species with closely related morphological characteristics. However, the identification and detection of *Phytophthora* spp. based on morphological characteristics is laborious, time consuming and expensive, and often requires specialized skill (Duncan & Cooke 2002). *Phytophthora* spp. usually require specific growth media and baiting techniques (Erwin & Riberio 1996), making it difficult to isolate *Phytophthora* spp. from soil or old infected plant material. Morphological classification relies on the production of pure cultures and induction of various diagnostic structures such as sporangia, chlamydospores, oogonia and antheridia. These key diagnostic structures can be variable within and between species (Erwin 1983) under different environmental conditions (Hendrix 1967, Brasier & Griffin 1979, Alizadeh & Tsao 1985).

Other limitations of using morphology to identify *Phytophthora* spp. include the difficulty of applying the type species concept as proposed by the International Code of Botanical Nomenclature (Brasier 1991). Preserving dried *Phytophthora* type cultures on infected host tissue and retaining the original

state of the culture remain a challege. There are also various unresolved taxonomic problems with the genus *Phytophthora*. Examples include the debates on the conspecificity of *P. nicotianae*, var. *nicotianae* and var. *parasitica* (Tucker 1931, Waterhouse 1963 and *P. crytogea* and *P. drechsleri* (Bumbieris 1974, Ho & Jong 1986). Species such as *P. megasperma* (Hansen 1991b, Hansen & Maxwell 1991a, Hansen *et al*. 2009) and *P. palmivora* have been reported as being species complexes.

6.1 Non-morphological classification methods

Given the shortcomings associated with the classification of *Phytophthora* spp. based on morphological characteristics alone, non-morphological methods are receiving increasing attention. Such methods include physiological and molecular techniques. Physiological characteristics that are useful for distinguishing subspecies and varieties within the genus include temperature-growth relationships, host specificity and chemical sensitivity (Shepherd 1976, Jung *et al.* 1999). Physiological characteristics are incorporated in taxonomic keys to support morphological variation between *Phytophthora* spp (Stamps *et al*. 1990). Each group within the genus *Phytophthora* consists of species with closely related physiological characteristics (Erwin & Riberio 1996). Response to different temperatures is one of the criteria used in taxonomic keys to distinguish between varieties within species (Newhook *et al*. 1978, Stamps *et al*. 1990, Erwin & Riberio 1996). *Phytophthora* spp. such as *P. infestans*, *P. mirabilis* and *P. phaseoli* are morphologically similar but can be distinguished on the basis of their host specificity (Brasier & Hansen. 1992). Hymexazol and malachite green have been successfully used to differentiate species and subspecies within Group I (Kennedy & Duncan 1995, Jung *et al.* 1999).

While the incorporation of physiological characteristics has helped to overcome some of the difficulties inherent in classification on purely morphological grounds, some taxonomic questions remain unresolved. Relatively, few *Phytophthora* spp. accurately match defined morphological groups (I–VI). The heterothallic species *P. capsici* and the homothallic *P. megasperma*, for example, exhibit complexity and appear to consist of clusters of biological species and discrete population units (Hansen *et al.* 1986). The term "biological species" refers to actual or potential interbreeding populations that are impossible to differentiate morphologically (Hawksworth *et al.* 1995). Brasier (1991) proposed a biological species concept in order to accommodate the changing status of species within species complexes. According to Brasier (1991) hybridization is another complicating factor that can hamper the morphological and physiological identification process. Part of the solution to these problems involves use of population analysis and complementary molecular methods.

Modern molecular techniques allow interpretation of useful DNA sequence information that is essential for the diagnosis and control of plant pathogens (Schena *et al*. 2004, Crous, 2005) Molecular tools, especially the Polymerase Chain Reaction (PCR), automated DNA sequencing and

bioinformatics have revolutionized the identification of many fungal pathogens, including *Phytophthora* (Cooke *et al.* 2000, Duncan & Cooke 2002, Kamoun *et al.* 2002). Universal primers published by White *et al.* (1990) fuelled the growth of fungal databases housing sequence data. The Internal Transcribed Spacer (ITS) regions of ribosomal RNA the ribosomal DNA has been used extensively for the identification of *Phytophthora* and other oomycete genera (Cooke *et al* 2000, de Cock & Lévesque 2004, Lévesque & de Cock 2004). It is now recognised that there are 10 ITS clades within *Phytophthora* which are not specifically correlated with particular unique morphological features Cooke *et al.* 2000). Accurate and rapid techniques to detect and identify *Phytophthora* spp. have been extensively investigated in recent years because of their importance as pathogens. Molecular techniques have been useful in confirming the validity of species within the genus *Phytophthora* and also provide a better resolution between morphology and genetic relationships amongst species (Martin *et al*. 2000). In the following sections, various *Phytophthora* detection and identification methods are discussed with special reference to *Phytophthora* disease management.

6.2 Methods for isolation, detection, and identification of Phytophthora

6.2.1 Serological methods

Phytophthora zoospores are attracted to a wide range of chemicals released from the roots of host plants (Deacon & Donaldson 1993). Serological methods can be used to monitor or detect *Phytophthora* spp. Antibodies derived from antisera against the whole organism are called polyclonal, and those derived antisera from single cloned cells are called monoclonal antibodies and thus are more specific than polyclonal antibodies (Hardham *et al*. 1986, 1991). Various serological methods have become valuable tools for research and commercial purposes. The advantages of serological methods over traditional baiting methods include rapid identification, low detection levels, detection before the disease symptoms are expressed by the host plant and accurate detection of *Phytophthora* regardless of the presence of other microorganisms (Erwin & Riberio 1996). However, the disadvantages of using serological methods are that they do not distinguish between dead and living propagules (Timmer *et al*. 1993) and may produce false positive results by detecting common antigens from related microorganisms. Thus they should always be used in conjunction with direct isolation methods (Macdonald *et al*. 1990). Robold & Hardham (1998) reported species specific monoclonal antibodies that react only with surface components on zoospores and cysts of *P. nicotianae*. Similarly, Hardham *et al*. (1986) and Ferraris *et al*. (2004) were also able to produce monoclonal antibodies binding only to cysts or zoospores of *P. cinnamomi* or and not to those of *P. parasitica* or *P. vignae*. Surface components of zoospores and cysts of *Phytophthora* and *Pythium* spp., have a range of specificities. Thus, they may have an important value in the taxonomy and phylogeny of species, as well as use in disease diagnosis (Hardham *et al*. 1994).

6.2.2 DNA probe detection methods

Cloned DNA fragments can be used as species-specific probes to detect *Phytophthora* spp. Goodwin *et al*. (1989, 1990b) used DNA probes to detect and identify *P. parasitica* and *P. citrophthora* among a range of different species. They also used DNA hybridization probes to detect *P. parasitica* directly from plant tissue (Goodwin *et al.* 1990a). Judelson & Messenger-Routh (1996) used a cloned DNA fragment of *P. cinnamomi* to quantitatively detect the pathogen. A number of DNA sequences have been generated through phylogenetic studies of *Phytophthora* and related genera sequence data have become available making it possible to design specific primers (Briard *et al.* 1990, Cooke *et al.* 1996, Crawford *et al.*1996, Lee & Taylor 1992). Examples of primer hybridization probes for *Phytophthora* spp. have been reported by Lee & Taylor (1992) and Lee *et al*. 1993).

6.2.3 Multigene phylogeny of *Phytophthora* species

Molecular studies based on single gene regions, especially the ITS region has been used extensively to infer phylogenetic relationships between species within the genus *Phytophthora* (Cooke *et al*. 2000, Martin & Tooley 2003, Blair *et al*. 2008). The ITS region, in particular is a widely method used routinely for identification of *Phytophthora* spp. (Drenth 2006). Modification of the universal ITS1 or ITS2 primers has been used in designing species-specific probes for detecting *P. citricola* in inoculated plants (Schubert *et. al*. 1999) or used in combination with the ITS for designing species-specific probes (Lee *et al.*1993, Coelho *et al.* 1997, Shen *et al.* 2005). Many species however, remain incompletely described and require more thorough molecular analysis (Brasier & Hansen 1992).

A multiple gene phylogeny based on combined nuclear DNA (translation elongation factor 1α; β-tubulin) and mitochondrial DNA (cytochrome c oxidase subunit 1; NADH dehydrogenase subunit 1) sequence data has been reported for the genus *Phytophthora* (Kroon *et al*. 2004). Recently, Schena & Cooke (2006) also reported multigene phylogeny of *Phytophthora* spp. based on intergenic spacer (IGS) regions of rDNA (rDNA-IGS), ras-related protein (Ypt1) gene and four regions of mitochondrial DNA (mt-IGS). Blair *at al*. (2008) produced a phylogeny based on 7 loci derived from the complete genome sequence of *P. ramorum, P. sojae*, and *P. infestans.* The resultant phylogeny is remarkably similar to that obtained from ITS sequence data alone. A number of species-specific molecular markers have been developed for *Phytophthora* spp.

6.2.4 Protein electrophoresis

Protein electrophoresis differentiates species on the basis of the protein profiles generated when mycelial proteins are dispersed into a medium and subjected to an electric current. Early application of

this technique in *Phytophthora* systematics was pioneered by Clare & Zentmyer (1966). Since then, protein electrophoresis has proven useful in distinguishing *Phytophthora* spp. (Boccas & Zentmyer 1976, Boccas 1981, Erselius & de Vallavieille 1984, Erselius & Shaw 1982, Bielenin *et al.* 1988, Chowdappa & Chandramohanan 1995) and resolving conspecific species (Bielenin *et al.* 1988, Masago *et al.* 1989) and morphospecies within *P. megasperma* species complex (Irwin & Dale 1982, Hansen *et al.* 1986). Protein electrophoresis is generally used to confirm species identification and thus provide support in cases where morphological data is not clear (Erwin & Riberio 1996). However, this technique generates a large number of bands that are often difficult to score and biosynthesis of some proteins is not constant (Förster & Coffey 1991).

6.2.5 Isozymes

Isozymes are alternative forms of an enzyme that have the same catalytic function (Spielman 1991). Isozymes can also be used to differentiate species (Latorre *et al.* 1995, Mills *et al*. 1991, Oudemans & Coffey 1991a, 1991b, Oudemans *et al*. 1994), biotypes (Nygaard *et al.* 1989) or prove conspecificity (Oudemans & Coffey 1991c, Mchau & Coffey 1994b) within the genus *Phytophthora.* However, they are generally used to study the genetic diversity within population of various *Phytophthora* spp. (Micales *et al*. 1986, Old *et al*. 1984, 1988, Goodwin 1997, Linde *et al.* 1997). Isozymes are less complex than protein profiles and are easy to differentiate and interpret. Isozymes represent the end-products of specific gene functions.

6.2.6 Restriction Fragment Length Polymorphisms (RFLPs)

Mitochondrial DNA (mtDNA) or chromosomal DNA (chrDNA) isolated from the species of interest is digested with restriction endonuclease enzymes, which cut the DNA at specific sites resulting in fragments of varying lengths. The size of the resulting fragments is visually estimated using molecular markers. *Phytophthora* spp. produce different fragment profiles that can be used to distinguish them. RFLPs are routinely used to study the genetic relationship between biotypes and species in the genus *Phytophthora* (Förster *et al.* 1987, 1988 1989, Förster & Coffey 1991). RFLPs are also useful in differentiating between morphologically distinct *Phytophthora* spp. (Panabiéres *et al.* 1989, Förster & Coffey 1991, Mills *et al.* 1991, de Cock *et al.* 1996) or similar species (Förster, *et al.* 1990b, Mirabolfathy *et al.* 2001). Other applications of RFLPs include population studies of pathogen races (Drenth *et al.* 1996, Lebreton & Andrivon 1998, Ordonez *et al.* 2000, Purvis *et al.* 2001) and pathogen populations from different host and locations (Linde *et al.* 1999a) and represent products of fuctional genes.

6.2.7 Random Amplified Polymorphic DNA (RAPDs)

RAPD markers represent a technique that involves random amplification of DNA of a chosen organism using short oligonucleotide primers. The resulting products are separated by electrophoresis and visually inspected and analysed (Karp *et al*. 1996). RAPDs have been used extensively in *Phytophthora* taxonomy to study the genetic variation within and between species. Examples include studies conducted by Linde *et al*. (1999a), Nyasse *et a*l. (1999) and Purwantara *et al*. (2001) who respectively compared the genetic diversity of *P. cinnamomi*, *P megakarya* and *P. clandestina* isolates from different geographic origins. RAPDs have been successfully used to differentiate between pathogen races (Chang *et al*. 1996, Förster, Tyler & Coffey 1994, Meng *et al*. 1999, Punja *et al*. 1998), morphological groups (Cooke *et al*. 1996, Jung *et al*. 1999, Elena & Paplomatas 1999, Goodwin *et al*. 1999) and to compare isolates of the same species from different hosts (Lilja *et al*. 1998) or geographic origins (Cooke *et al*. 1999).

6.2.8 Amplified Fragment Length Polymorphisms (AFLPs)

AFLP is a fingerprinting method involving restriction digests of genomic DNA, ligation of oligonucleotide adapters and selective amplification products of restriction fragments (Vos *et al.* 1995). AFLP can be a useful tool in discriminating *Phytophthora* spp., especially when used to compliment other molecular techniques (Mirabolfathy *et al.* 2001, Werres *et al.* 2001). AFLPs have been used successfully in detecting genetic variation in fungi (Majer *et al.* 1996) and in characterizing hybrid isolates of *P. nicotianae* and *P. cactorum* (Bonants *et al.* 2000). However, they have not been extensively used in *Phytophthora* taxonomy.

7 Conclusion

South Africa has inadequate natural timber resources to supply industrial demand and thus depends on plantations of non-native species such as eucalypts. The history of eucalypt plantations in South Africa dates back more than one hundred years. Improved *E. grandis* and it's various hybrids and clones are the most commonly planted species. During the past decades, forestry has been expanding towards marginal areas which are unsuitable for *E. grandis,* consequently there is an increased demand for altenative species.

Small areas of cold-tolerant eucalypts are planted at high altitude in South Africa. However, the afforestation potential of the majority of these species is limited by the root rot complex involving *P.*

cinnamomi. The role of *P. cinnamomi* in causing disease of many forest species has been studied extensively. However, very little is known about the role of other *Phytophthora* spp. associated with the die-back of cold-tolerant eucalypts in South Africa.

The taxonomy of the genus *Phytophthora* has undergone extensive revision. This revision commenced with the reclassification of Oomycetes and closely related genera under a recently described kingdom Chromista. For years scientists have relied on traditional taxonomic keys to identify *Phytophthora* spp. However, inherent problems associated with variation of morphological features within and between species have resulted in some areas of confused taxonomy. The advent of various molecular tools, especially comparisons based on sequences of the ITS regions of the rDNA operon has advanced the taxonomy of the genus *Phytophthora*. Many species thought to be different or similar have since been found to be either co specific or belonging to species complexes respectively. In addition, a number of hybrid *Phytophthora* spp. have emerged in many parts of the world, resulting in wider host range and further taxonomic confusion. Understanding the genetic diversity of *Phytophthora* spp. remains an area deserving considerably more study.

The expression of *Phytophthora* related disease symptoms differs significantly between native eucalypt forests and man-made plantations. Plantations are established in monoculture and are genetically uniform and more prone to invasion by aggressive pathogens such *P. cinnamomi*. The development of *Phytophthora*-related disease symptoms in eucalypt plantations depends on a number of constantly changing abiotic and biotic factors. These factors are strongly influenced by the species involved and its pathogenicity, level on infestation and prevailing environmental conditions. *Eucalyptus* spp. also differ significantly in their tolerance to *Phytophthora* spp. Species belonging to the sub-genus *Monocalyptus* are generally more susceptible to *Phytophthora* root rot than those belonging to the *Symphyomyrtus*.

Disease management strategies used in reducing the impact of *Phytophthora* root rot in *Eucalyptus* plantations are primarily preventative in nature because chemical control is not economical. Disease control strategies mainly involve resistance breeding, use of various silvicultural practices and quarantine. Accurate identification of the pathogen (s) involved as well as sound understanding of the population structure and the biology of the pathogen are fundamental to successful breeding programme. With little being known about *Phytophthora* spp. associated with eucalypts in South Africa the following work addressed the taxonomy of *Phytophthora* spp. and interactions with eucalypt hosts.

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